REMARKS

The Examiner is respectfully requested to provide a Notice of References Cited Form PTO-892 which lists Clive et al. which is applied in a prior art rejection in the Office Action.

Regarding Paragraph No. 1 at the top of page 2 of the Office Action, the intended specification amendment was for page 12, line 15 (not line 26) of the specification. However, the intended specification amendment is not necessary. Please therefore disregard such specification amendment.

35 USC 112 Rejections

Claims 1 to 8 were rejected under 35 USC 112, first paragraph, for the reasons set forth in Paragraph No. 5 at the top of page 3 of the Office Action for allegedly introducing "new matter".

Claims 1 to 8 were rejected under 35 USC 112, second paragraph, as being indefinite for the reasons a. and b. set forth in Paragraph No. 7 at the bottom of page 3 of the Office Action.

It is noted that the term "micro detection field" is clearly defined on page 16, lines 13 to 25 in the specification.

The term "migration distance" is no longer recited in the claims.

Claims 3 and 4 were amended to avoid the 35 USC 112, first paragraph rejection.

The amendment to claim 3 is supported in the specification on page 5, lines 11 to 12.

The amendment to claim 4 is supported in the specification on

page 5, lines 13 to 16.

Enclosed is a MARKED UP VERSION OF THE AMENDMENTS TO THE CLAIMS.

It is respectfully submitted that the present claims comply with all the requirements of 35 USC 112.

With respect of Rule 116, entry of the above amendments is respectfully requested, since the amendments are in reply to 35 USC 112 rejections set forth in the final rejection and because the amendments place the application in better form for an appeal, should an appeal be necessary.

Presently Claimed Invention

The presently claimed invention concerns a method of analyzing a target nucleic acid by applying a nucleic acid amplification reaction to a test solution. The method comprises:

(a) performing a nucleic acid amplification reaction of the target nucleic acid to provide nucleic acid amplification products including amplified nucleic acid in a test solution containing a forward primer and a reverse primer, a substrate comprising nucleotides, wherein at least one of the nucleotides is labeled with a marker molecule capable of generating a detectable signal, a nucleic acid polymerase, and a target nucleic acid molecule;

(b) measuring a signal from the marker molecule in the test solution after initiation of the nucleic acid amplification reaction;

(c) evaluating the mobility of the amplified nucleic acid which is labeled with the marker molecule, in the test solution on

the basis of the signal detected; and

(d) quantifying the target nucleic acid molecule on the basis of evaluation results.

Prior Art Rejection

Claims 1 to 8 were rejected under 35 USC 103 as being unpatentable over Mullis et al. USP 4,965,188 in view of Clive et al., J. Neurosci. Methods, 1998, Vol. 81, pp. 25-34 for the reasons set forth in Paragraph Nos. 3 and 4 on pages 2 to 3 of the April 25, 2001 Office Action.

The previous Office Action of August 3, 2000 admitted that Mullis et al. do not disclose quantifying the amplified target nucleic acid based on evaluation results.

The previous Office Action of August 3, 200 also admitted that Mullis et al. do not disclose removing labeled substrates which are not incorporated in the amplified nucleic acid

In Paragraph No. 3 near the bottom of page 2 of the April 25, 2001 Office Action, it was asserted that "It is unclear how the migration distance of the marker molecule integrated in the amplified nucleic acid sequence is monitored and determined in accordance with an autocorrelation based upon the description of the specification".

The term "migration distance" in the claims has been changed hereinabove to --fluctuation motion--, based on the description on page 13 in the specification.

It is respectfully submitted that the step of monitoring and determining (objected to as being vague by the Examiner) would be

clearly understood by those of ordinary skill in the art on the basis of the description on page 5, lines 9 to 22 in the specification and the paper cited in the specification on page 5, lines 21 to 22 (Kinjo M., Rigler, R., Nucleic Acids Research, 23, 1795-1799, (1995)).

In addition, workers having ordinary skill in the art should understand such monitoring and determining step with reference to page 36, line 23 to page 39, line 26 of the specification under the headings of "(2) FCS measurement" and "(3) Calculation of measurement region".

Enclosed is a copy of the above identified Kinjo et al. paper.

Moreover, the principles of fluorescence correlation spectroscopy ("FCS") are outlined hereinbelow.

FCS (Fluorescence Correlation Spectroscopy)

When molecules bind to each other, the resultant molecular weight increases. Conversely, when a molecule is cleaved, the resultant molecular weight decreases. If molecules present in a solution are subjected to the aforementioned situation, the Brownian movement of the molecules increases or decreases depending upon the size of the molecule. Furthermore, when molecules bind to each other, the number of molecules decreases. Conversely, when a molecule is cleaved, the number of molecules increases. Such a change in movement (action) of molecules and the number of molecules can be measured (monitored) by FCS (Fluorescence Correlation Spectroscopy) based on the fluctuation of fluorescence intensity of a fluorescent dye.

A basic feature of FCS resides in that the concentration and intermolecular action of fluorescent molecules contained in a homogenous solution can be monitored in real time without any physical separation step, based on the "fluctuation" of light derived from the Brownian movement of several fluorescent molecules (on average) present in a small region under the field of view of a microscope. Since FCS detects a free movement of molecules in a solution as discussed above, it has been expected to be applied to a wide variety of research.

In the FCS measurement system, a confocal optical system using a laser excitation is employed in a sample measurement portion. Fluorescent light emitted from the sample measurement portion is captured by a detector. The captured data is sent from the detector to a digital correlation analyzer, in which data is recorded and analyzed. The confocal optical system has a feature in that a laser beam (excitation light) is converged to a single point in a sample solution, and fluorescence emitted from the point is captured by a detector system. However, it is difficult to measure the fluorescent light at a point of a solution, so that fluorescence in a cylindrical region is actually measured. The cylindrical region has, for example, a diameter of about 400 nm, an axial length of about 2000 nm and a volume which is in the order of a femto-liter (10^{-15}L).

In addition, since the FCS measurement region resides in a solution, fluorescent molecules present in the measurement region always move in accordance with Brownian movement. Therefore, the number of molecules present in a predetermined measurement region

is not constant and fluctuates around a certain value (called "fluctuation in number").

Furthermore, in accordance with the "fluctuation in number", the measured fluorescence intensity fluctuates (called "fluctuation of fluorescence intensity"). Therefore, if the "fluctuation of fluorescence intensity" is measured, it is possible to obtain data on the relationship between the diffusion speed and the number of molecules.

The Present Claims Distinguish Over the Cited Prior Art

It is respectfully submitted that one of ordinary skill in the art would not combine Mullis et al. and Clive et al. to attempt to arrive at the presently claimed invention.

Assuming *arguendo*, however, that the references are combinable, if Mullis et al. is combined with Clive et al., it would not be possible to detect an amplified nucleic acid sequence without using electrophoresis. In contrast thereto, in the presently claimed invention, a specific nucleic acid sequence can be detected and quantified by monitoring the mobility of marker molecules integrated in an amplified nucleic acid sequence (see applicant's claim 1). Therefore, it is not necessary to perform a step of electrophoresis on the amplified nucleic acid sequence.

In addition, in the presently claimed invention, a series of requisite operations (see claim 1, steps (a) to (d)) are performed in a test solution. In contrast to the presently claimed invention, the techniques disclosed by Mullis et al. and Clive et al. are directed to detecting movement of a nucleic acid sequence in a

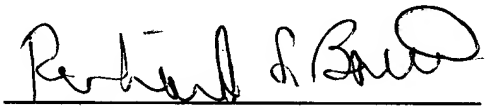
solid phase by electrophoresis. Hence, even if Mullis et al. and Clive et al. (both perform an electrophoretic operation in a solid phase) are combined, the presently claimed invention (involving requisite operations in a liquid phase) would not be arrived at.

It is therefore respectfully submitted that applicant's claimed invention is not rendered obvious over the references, either singly or combined in the manner relied upon in the Office Action in view of the distinctions discussed hereinabove. It is furthermore submitted that there are no teachings in the references to combine them in the manner relied upon in the Office Action.

Reconsideration is requested. Allowance is solicited.

If the Examiner has any comments, questions, objections or recommendations, the Examiner is invited to telephone the undersigned at the telephone number given below for prompt action.

Respectfully submitted,



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Enclosures: (1) MARKED UP VERSION OF THE AMENDMENTS TO THE CLAIMS
(2) copy of Kinjo et al., Nucleic Acid Research, 23, 1795-1799

MARKED UP VERSION OF THE AMENDMENTS TO THE CLAIMS

3. (Twice Amended) A method according to claim 2, wherein, in the measurement step, a [migration distance] fluctuation motion of the amplified nucleic acid labeled with the marker molecule [within a predetermined time interval is measured for a plurality of times] in fluid is measured.

4. (Twice Amended) A method according to claim 3, wherein the evaluation step [includes a step of converting a change of the migration distance into statistical data, on the basis of a plurality of measurement data] comprises a measurement which is affected by fluorescence correlation spectroscopy.